GEN-PROBE®

APTIMA®

APTIMA® HPV Assay

For *in vitro* diagnostic use.

General Information

Intended Use

The APTIMA HPV Assay is an *in vitro* nucleic acid amplification test for the qualitative detection of E6/E7 viral messenger RNA (mRNA) from 14 high-risk types of human papillomavirus (HPV) in cervical specimens. The high-risk HPV types detected by the assay include: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. The APTIMA HPV Assay does not discriminate between the 14 high-risk types. Cervical specimens in ThinPrep Pap Test vials containing PreservCyt Solution and collected with broom-type or cytobrush/spatula collection devices* may be tested with the APTIMA HPV Assay. The assay is used with the TIGRIS DTS System.

The use of the test is indicated:

1. To screen patients 21 years and older with atypical squamous cells of undetermined significance (ASC-US) cervical cytology results to determine the need for referral to colposcopy. The results of this test are not intended to prevent women from proceeding to colposcopy.
2. In women 30 years and older, the APTIMA HPV Assay can be used with cervical cytology to adjunctively screen to assess the presence or absence of high-risk HPV types. This information, together with the physician's assessment of cytology history, other risk factors, and professional guidelines, may be used to guide patient management.

* Broom-type device (e.g., Wallach Pipette) or endocervical brush/spatula.

Warning: *This assay is not intended for use as a screening device for women under age 30 with normal cervical cytology.*

Warning: *The APTIMA HPV Assay is not intended to substitute for regular cervical cytology screening.*

Warning: *Detection of HPV using the APTIMA HPV Assay does not differentiate HPV types and cannot evaluate persistence of any one type.*

Warning: *The use of this assay has not been evaluated for the management of HPV vaccinated women, women with prior ablative or excisional therapy, hysterectomy, who are pregnant, or who have other risk factors (e.g., HIV+, immunocompromised, history of sexually transmitted infection).*

Warning: *The APTIMA HPV Assay is designed to enhance existing methods for the detection of cervical disease and should be used in conjunction with clinical information derived from other diagnostic and screening tests, physical examinations, and full medical history in accordance with appropriate patient management procedures.*

Summary and Explanation of the Test

Cervical cancer is one of the most common female cancers in the world. HPV is the etiological agent responsible for more than 99% of all cervical cancers.[1, 2, 3] HPV is a common sexually transmitted DNA virus comprised of more than 100 genotypes.[1]

Only approximately 14 of the genotypes are considered pathogenic or high-risk for cervical disease.[4] Multiple studies have linked genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 to disease progression.[2, 5, 6] Patients with a persistent infection with one of these types have an increased risk for developing severe dysplasia or cervical carcinoma.[4, 7]

The HPV viral genome is a double-stranded circular DNA approximately 7900 base pairs in length. The genome has eight overlapping open reading frames. There are six early (E)

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genes, two late (L) genes, and one untranslated long control region. The L1 and L2 genes encode the major and minor capsid proteins. Early genes regulate HPV viral replication. The E6 and E7 genes of high-risk HPV genotypes are known oncogenes. Proteins expressed from E6-E7 polycistronic mRNA alter cellular p53 and retinoblastoma protein functions, leading to disruption of cell-cycle check points and cell genome instability.[1,8]

HPV infections are very common and most women will clear HPV infections within 6 to 12 months.[2,9] The presence of HPV nucleic acid does not mean that cervical dysplasia or cervical cancer is present. However, an effective approach for detection of cervical disease is to target those oncogenic elements of HPV that foster persistent viral infection and cellular transformation.[10]

Principles of the Procedure

The APTIMA HPV Assay involves three main steps, which take place in a single tube: target capture; target amplification by Transcription-Mediated Amplification (TMA);[11] and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).[12] The assay incorporates an internal control to monitor nucleic acid capture, amplification, and detection, as well as operator or instrument error.

Specimens are transferred to a tube containing specimen transport media (STM) that lyses the cells, releases the mRNA, and protects it from degradation during storage. When the APTIMA HPV Assay is performed, the target mRNA is isolated from the specimen by use of capture oligomers that are linked to magnetic microparticles. The capture oligomers contain sequences complementary to specific regions of the HPV mRNA target molecules as well as a string of deoxyadenosine residues. During the hybridization step, the sequence-specific regions of the capture oligomers bind to specific regions of the HPV mRNA target molecule. The capture oligomer-target complex is then captured out of solution by decreasing the temperature of the reaction to room temperature. This temperature reduction allows hybridization to occur between the deoxyadenosine region on the capture oligomer and the poly-deoxythymidine molecules that are covalently attached to the magnetic particles. The microparticles, including the captured HPV mRNA target molecules bound to them, are pulled to the side of the reaction tube using magnets and the supernatant is aspirated. The particles are washed to remove residual specimen matrix that may contain amplification inhibitors.

After target capture is complete, the HPV mRNA is amplified using TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy of the target mRNA sequence containing a promoter sequence for T7 RNA polymerase. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Detection of the amplicon is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The Selection Reagent differentiates between hybridized and unhybridized probes by inactivating the label on the unhybridized probes. During the detection step, light emitted from the labeled RNA-DNA hybrids is measured as photon signals called Relative Light Units (RLU) in a luminometer. Final assay results are interpreted based on the analyte signal-to-cutoff (S/CO).

Internal Control is added to each reaction via the Target Capture Reagent. The Internal Control monitors the target capture, amplification, and detection steps of the assay. Internal Control signal in each reaction is discriminated from the HPV signal by the differential

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kinetics of light emission from probes with different labels.[13] Internal Control-specific amplicon is detected using a probe with a rapid emission of light (flasher). Amplicon specific to HPV is detected using probes with relatively slower kinetics of light emission (glower). The Dual Kinetic Assay (DKA) is the method used to differentiate between the signals from the flasher and glower labels.[13]

Warnings and Precautions

A. For *in vitro* diagnostic use.

For additional specific warnings and precautions related to instrumentation refer to the TIGRIS DTS System Operator's Manual.

Laboratory Related

B. Use only supplied or specified disposable laboratory ware.

C. Use routine laboratory precautions. Do not eat, drink, or smoke in designated work areas. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

D. **Warning: Irritants and Corrosives:** Avoid contact of Auto Detect 1 and Auto Detect 2 with skin, eyes and mucous membranes. If these fluids come into contact with skin or eyes, wash the affected area with water. If these fluids spill, dilute the spill with water before wiping it dry.

E. Work surfaces, pipettes, and other equipment must be regularly decontaminated with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Refer to *Procedural Notes* for more information.

Specimen Related

F. Test only the indicated specimen type. The APTIMA HPV Assay has only been validated for use with cervical specimens collected in PreservCyt solution using a broom-type or cytobrush/spatula collection device.

G. Collect cervical specimens in ThinPrep Pap Test vials containing PreservCyt Solution with broom-type or cytobrush/spatula collection devices according to the manufacturer's instructions. Aliquots subsequently removed from the PreservCyt vial for testing by the APTIMA HPV Assay should be processed using only the APTIMA Specimen Transfer Kit.

H. PreservCyt solution specimens were evaluated for use with the APTIMA HPV Assay after processing on the ThinPrep 2000 processor. Specimens processed using the ThinPrep 3000 Processor or other instruments have not been evaluated.

I. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

J. Expiration dates listed on specimen transfer kits and tubes pertain to the transfer site and not the testing facility. Specimens transferred any time prior to these expiration dates are valid for testing provided they have been transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

K. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this procedure.

L. Avoid cross-contamination during the specimen handling steps. Ensure that specimen containers do not contact one another, and discard used materials without passing over open containers. Change gloves if they come in contact with specimen.

M. Upon piercing, liquid can discharge from tube caps under certain conditions. Refer to the *Test Procedure* for more information.

Assay Related

N. Store reagents at the specified temperatures. Performance of the assay may be affected by use of improperly stored reagents.

O. Avoid microbial and ribonuclease contamination of reagents.

P. Do not use kit after its expiration date.

Q. Do not interchange, mix, or combine reagents from kits with different lot numbers.

R. Tips with hydrophobic plugs must be used.

Reagent Storage and Handling Requirements

Do not use reagents beyond the expiration date indicated on the vials. See below for additional storage instructions.

A. The following reagents are stored at 2°C to 8°C (refrigerated) upon receipt:

HPV Amplification Reagent

HPV Enzyme Reagent

HPV Probe Reagent

HPV Internal Control Reagent

HPV Positive Calibrators and Negative Calibrators

HPV Positive Controls and Negative Controls

B. The following reagents are stored at room temperature (15°C to 30°C):

HPV Amplification Reconstitution Solution

HPV Enzyme Reconstitution Solution

HPV Probe Reconstitution Solution

HPV Target Capture Reagent

HPV Selection Reagent

Wash Solution

Oil Reagent

Buffer for Deactivation Fluid

Auto Detect Reagent 1

Auto Detect Reagent 2

APTIMA System Fluid Preservative

C. After reconstitution, the following reagents are stable for 30 days when stored at 2°C to 8°C:

HPV Enzyme Reagent

HPV Amplification Reagent

HPV Probe Reagent

D. Working Target Capture Reagent (wTCR) is stable for 30 days when stored at 15°C to 30°C. Do not refrigerate.

E. Discard any unused reconstituted reagents and wTCR after 30 days or after the expiration date of the Master Lot, whichever comes first.

F. The APTIMA HPV Assay reagents are stable for a cumulative of 48 hours when stored on-board the TIGRIS DTS System.

G. The Probe Reagent and Reconstituted Probe Reagent are photosensitive. Store the reagents protected from light.

H. Do not freeze reagents.

Specimen Collection and Storage

A. Specimen collection and processing:

1. Collect cervical specimens in ThinPrep Pap Test vials containing PreservCyt Solution with broom-type or cytobrush/spatula collection devices according to the manufacturer's instructions.
2. If the ThinPrep Aliquot Removal procedure will be used, refer to the ThinPrep 2000 or ThinPrep 3000 Processor Operator's Manual – Addendum for instructions on aliquot removal. Transfer 1 mL of the removed aliquot into an APTIMA Specimen Transfer tube according to the instructions in the APTIMA Specimen Transfer Kit package insert.
3. If testing the specimen after processing using the ThinPrep 2000 Processor, process the PreservCyt Solution liquid cytology specimen in accordance with the ThinPrep 2000 Processor Operator's Manual and the APTIMA Specimen Transfer Kit package insert. Transfer 1 mL of the fluid remaining in the PreservCyt Solution vial into an APTIMA Specimen Transfer tube according to the instructions in the APTIMA Specimen Transfer Kit package insert.

B. Transport and storage before testing:

1. PreservCyt Solution specimens should be transported and stored at 2°C to 30°C, with no more than 30 days at temperatures above 8°C.
2. PreservCyt specimens should be transferred to an APTIMA Specimen Transfer tube within 105 days of collection.
3. PreservCyt Solution specimens transferred to an APTIMA Specimen Transfer tube may be stored at 2°C to 30°C for up to 60 days.
4. If longer storage is needed, the PreservCyt Solution specimen or the PreservCyt Solution specimen diluted into the Specimen Transfer tube may be stored at -20°C for up to 24 months.

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C. Specimen storage after testing:

1. Specimens that have been assayed must be stored upright in a rack.
2. Specimen tubes should be covered with a new, clean plastic or foil barrier.
3. If assayed specimens need to be frozen or shipped, remove penetrable cap and place new non-penetrable caps on the specimen tubes. If specimens need to be shipped for testing at another facility, specified temperatures must be maintained. Prior to uncapping previously tested and recapped specimens, tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube.

Note: *National, regional, and local requirements for packaging must be met when specimens are transported by common land and air carriers.*

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TIGRIS DTS System

Reagents and Materials Provided

APTIMA HPV Assay Kit for TIGRIS DTS System, 250 tests, Cat. No. 303012 (4 boxes)

Calibrators and Controls may be purchased separately. See individual catalog numbers below.

APTIMA HPV Refrigerated Box
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
A	**HPV Amplification Reagent** *Non-infectious nucleic acids dried in buffered solution containing < 5% bulking agent.*	1 vial
E	**HPV Enzyme Reagent** *Reverse transcriptase and RNA polymerase dried in HEPES buffered solution containing < 10% bulking reagent.*	1 vial
P	**HPV Probe Reagent** *Non-infectious chemiluminescent DNA probes (< 500 ng/vial) dried in succinate buffered solution containing < 5% detergent.*	1 vial
IC	**HPV Internal Control Reagent** *Non-infectious RNA Transcript in buffered solution containing < 5% detergent.*	1 vial

APTIMA HPV Room Temperature Box
(store at 15°C to 30°C upon receipt)

Symbol	Component	Quantity
AR	**HPV Amplification Reconstitution Solution** *Aqueous solution containing preservatives.*	1 vial
ER	**HPV Enzyme Reconstitution Solution** *HEPES buffered solution containing a surfactant and glycerol.*	1 vial
PR	**HPV Probe Reconstitution Solution** *Succinate buffered solution containing < 5% detergent.*	1 vial
S	**HPV Selection Reagent** *600 mM borate buffered solution containing surfactant.*	1 vial
TCR	**HPV Target Capture Reagent** *Non-infectious nucleic acid in a buffered solution containing solid phase (< 0.5 mg/mL).*	1 vial
	Reconstitution Collars	3
	Master Lot Barcode Sheet	1 sheet

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APTIMA HPV Calibrators Box (Cat. No. 303010)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
PCAL	**HPV Positive Calibrator** *Non-infectious HPV 16 in vitro transcript at 1000 copies per mL in a buffered solution containing < 5% detergent.*	5 vials
NCAL	**HPV Negative Calibrator** *Buffered solution containing < 5% detergent.*	5 vials

APTIMA HPV Controls Box (Cat. No. 303011)
(store at 2°C to 8°C upon receipt)

Symbol	Component	Quantity
PC	**HPV Positive Control** *Lysed, inactivated HPV Negative and HPV Positive cultured cells at 25 cells per mL in a buffered solution containing < 5% detergent.*	5 vials
NC	**HPV Negative Control** *Lysed, inactivated HPV Negative cultured cells in a buffered solution containing < 5% detergent.*	5 vials

Materials Required But Provided Separately

Note: *Materials available from Gen-Probe have catalog numbers listed, unless otherwise specified.*

	Cat. No.
TIGRIS DTS System	105118
APTIMA Assay Fluids Kit *(APTIMA Wash Solution, APTIMA Buffer for Deactivation Fluid, and APTIMA Oil Reagent)*	302382
APTIMA Auto Detect Kit	301048
APTIMA System Fluid Preservative Kit	302380
Micropipettor, 1000 µL RAININ PR1000	104216
Tips, 1000 µL P1000	105049
Tips, 1000 µL conductive, liquid sensing	10612513 (Tecan)
MTUs	104772-02
MTU/Tiplet Waste Bag Kit	900907
MTU Waste Deflectors	900931
MTU Waste Cover	105523
APTIMA Specimen Transfer Kit *for use with ThinPrep liquid cytology specimens*	301154C
APTIMA Penetrable Caps	105668
Replacement non-penetrable caps	103036A
Bleach, minimum 5% or 0.7 M sodium hypochlorite solution	
Water for the TIGRIS DTS System *consult the TIGRIS DTS System Operator's Manual for specifications*	
Disposable gloves	

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Test Procedure

Note: *See TIGRIS DTS System Operator's Manual for additional TIGRIS DTS System procedural information.*

A. Work Area Preparation

Clean work surfaces where reagents will be prepared. Wipe down work surfaces and pipettors with 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution. Allow sodium hypochlorite solution to contact surfaces and pipettors for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the reagents will be prepared with clean, plastic-backed absorbent laboratory bench covers.

B. Reagent Preparation of a New Kit

Note: *Reagent Reconstitution should be performed prior to beginning any work on the TIGRIS DTS System.*

1. To reconstitute Amplification, Enzyme, and Probe Reagents, combine the bottles of lyophilized reagent with the reconstitution solution. If refrigerated, allow the reconstitution solutions to reach room temperature before use.
   a. Pair each reconstitution solution with its lyophilized reagent. Ensure that the reconstitution solution and lyophilized reagent have matching label colors before attaching the reconstitution collar.
   b. Check the lot numbers on the Master Lot Barcode Sheet to ensure that the appropriate reagents are paired.
   c. Open the lyophilized reagent vial and firmly insert the notched end of the reconstitution collar into the vial opening (Figure 1, Step 1).
   d. Open the matching reconstitution solution, and set the cap on a clean, covered work surface.
   e. While holding the solution bottle on the bench, firmly insert the other end of the reconstitution collar into the bottle opening (Figure 1, Step 2).
   f. Slowly invert the assembled bottles. Allow the solution to drain from the bottle into the glass vial (Figure 1, Step 3).
   g. Gently swirl the solution in the vial to mix. Avoid creating foam while swirling the vial (Figure 1, Step 4).
   h. Wait for the lyophilized reagent to go into solution, then invert the assembled bottles again, tilting at a 45° angle to minimize foaming (Figure 1, Step 5). Allow all of the liquid to drain back into the plastic bottle.
   i. Remove the reconstitution collar and vial (Figure 1, Step 6).
   j. Recap the bottle. Record operator initials and the reconstitution date on the label (Figure 1, Step 7).
   k. Discard the reconstitution collar and vial (Figure 1, Step 8).

Warning: *Avoid creating foam when reconstituting reagents. Foam compromises the level-sensing in the TIGRIS DTS System.*

Note: *Thoroughly mix Amplification, Enzyme, Probe, and Selection Reagents by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents.*

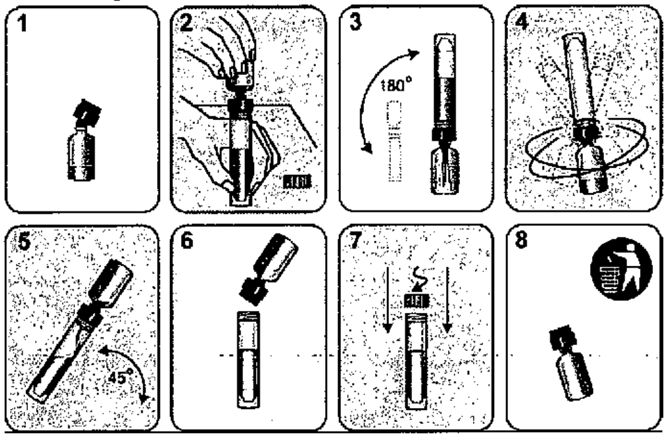

Figure 1. TIGRIS DTS System reconstitution process

2. Prepare the working Target Capture Reagent (wTCR):
   a. Pair the appropriate bottles of TCR and Internal Control (IC).
   b. Check the reagent lot numbers on the Master Lot Barcode Sheet to make sure that the appropriate reagents are paired.
   c. Open the bottle of TCR, and set the cap on a clean, covered work surface.
   d. Open the bottle of IC and pour the entire contents into the bottle of TCR. Expect a small amount of liquid to remain in the IC bottle.
   e. Cap the bottle of TCR and gently swirl the solution to mix the contents. Avoid creating foam during this step.
   f. Record operator initials and the current date on the label.
   g. Discard the IC bottle and cap.
   h. Precipitate may form in wTCR which may yield invalid results due to volume verification errors. Precipitate may be dissolved by warming wTCR at 42°C to 60°C for up to 90 minutes. Allow the wTCR to equilibrate to room temperature prior to use. Do not use if precipitate persists.
3. Prepare Selection Reagent
   a. Check the reagent lot number on the Master Lot Barcode Sheet to make sure it belongs to the kit.
   b. The Selection Reagent may precipitate if the temperature of the laboratory falls below 15°C or if the Selection Reagent has inadvertently been stored at 2°C to 8°C. Warm the Selection Reagent at 60°C for up to 45 minutes to facilitate dissolution of precipitate. Gently mix the bottle every 5 to 10 minutes. Allow the Selection Reagent to equilibrate to room temperature prior to use. Do not use if precipitate or cloudiness persists.

Note: *Thoroughly mix by gently inverting all reagents prior to loading on the system. Avoid creating foam during inversion of reagents.*

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C. Reagent Preparation for Previously Reconstituted Reagents

1. Previously reconstituted Probe, Amplification, and Enzyme Reagents must reach room temperature (15°C to 30°C) prior to the start of the assay.
2. If reconstituted Probe Reagent contains precipitate that does not return to solution at room temperature, heat at a temperature that does not exceed 60°C for 1 to 2 minutes. Do not use if precipitate or cloudiness is present.
3. If wTCR contains precipitate, warm wTCR at 42°C to 60°C for up to 90 minutes. Allow the wTCR to equilibrate to room temperature prior to use. Do not use if precipitate persists.
4. If Selection Reagent contains precipitate, warm the Selection Reagent at 60°C for up to 45 minutes to facilitate dissolution of precipitate. Gently mix the bottle every 5 to 10 minutes. Allow the Selection Reagent to equilibrate to room temperature prior to use. Do not use if precipitate or cloudiness persists.
5. Thoroughly mix each reagent by gently inverting prior to loading on the system. Avoid creating foam during inversion of reagents.
6. Do not top off reagent bottles. The TIGRIS DTS System will recognize and reject bottles that have been topped off.

D. Sample Handling

1. Allow the samples (calibrators, controls, and specimens) to reach room temperature prior to processing.
2. **Do not vortex samples.**
3. Inspect sample tubes before loading into the racks:
   a. If a sample tube contains bubbles in the space between the liquid and the cap, centrifuge the tube for 5 minutes at 420 RCF to eliminate the bubbles.
   b. If an APTIMA Specimen Transfer tube has a lower volume than is typically observed when collection instructions have been followed, centrifuge the tube for 5 minutes at 420 RCF to ensure that no liquid is in the cap.

Note: *Failure to follow steps 3a – 3b may result in liquid discharge from the sample tube cap.*

E. System Preparation

Set up the system and worklist according to the instructions in the *TIGRIS DTS System Operator's Manual* and the *Procedural Notes* section below.

Procedural Notes

A. Calibrators

1. Each worklist must contain 3 replicates of the Negative Calibrator and Positive Calibrator. In order to work properly with the APTIMA HPV Assay Software, the Negative Calibrator must be in the first tube position of the first rack of the worklist and the Positive Calibrator must be in the second tube position of the first rack of the worklist.
2. Attempts to pipette more than three replicates from a calibrator tube can lead to insufficient volume errors.

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B. Controls

1. The APTIMA HPV Assay software requires beginning and end run controls. The Negative Control must be in the third tube position of the first rack and the second to last tube position of the last rack of the worklist. The Positive Control must be in the fourth tube position of the first rack and the last tube position of the last rack of the worklist.
2. Attempts to pipette more than once from a control tube can lead to insufficient volume errors.

C. Temperature

Room temperature is defined as 15°C to 30°C.

D. Glove Powder

As in any reagent system, excess powder on some gloves may cause contamination of opened tubes. Powderless gloves are recommended.

E. Rack Decontamination

Submerge the racks in 2.5% to 3.5% (0.35M to 0.5M) sodium hypochlorite solution, ensuring that they are covered by the solution. Keep the racks submerged for 10 minutes. Longer exposure will damage the racks. Rinse the racks thoroughly with water and place on a clean absorbent pad; allow the racks to air-dry thoroughly.

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Quality Control Procedures

A. Run Validity Criteria

The software automatically determines run validity. The software will invalidate a run if any of the following conditions occur:

1. More than one invalid Negative Calibrator replicate.
2. More than one invalid Positive Calibrator replicate.
3. An invalid Negative Control.
4. An invalid Positive Control.

A run may be invalidated by an operator if technical, operator, or instrument difficulties are observed and documented while performing the assay.

An invalid run must be repeated.

B. Calibrator Acceptance Criteria

The table below defines the RLU criteria for the Negative and Positive Calibrator replicates.

Negative Calibrator	
Analyte	*≥ 0 and ≤ 45,000 RLU*
Internal Control	*≥ 75,000 and ≤ 400,000 RLU*
Positive Calibrator	
Analyte	*≥ 480,000 and ≤ 1,850,000 RLU*
Internal Control	*≤ 450,000 RLU*

C. Internal Control Cutoff Calculation

The Internal Control cutoff is determined from the Internal Control (flasher) signal from the valid Negative Calibrator replicates.

IC Cutoff *= 0.5 x [mean IC RLU of the valid Negative Calibrator replicates]*

D. Analyte Cutoff Calculation

The analyte cutoff is determined from the analyte (glower) signal from the valid Negative Calibrator replicates as well as the analyte signal from the valid Positive Calibrator replicates.

Analyte Cutoff = *[mean analyte RLU of the valid Negative Calibrator replicates] + [0.09 x mean analyte RLU of the valid Positive Calibrator replicates]*

E. Analyte Signal to Cutoff (S/CO) Calculation

The analyte S/CO is determined from the analyte RLU of the test sample and the analyte cutoff for the run.

$$\textbf{Analyte S/CO} = \frac{\textit{test sample analyte RLU}}{\textit{analyte cutoff}}$$

F. Control Acceptance Criteria

The Negative Control must have a valid negative result (IC RLU ≥ IC cutoff and analyte S/CO < 0.50). The Positive Control must have a valid positive result (analyte S/CO ≥ 0.50).

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Test Interpretation

Assay test results are automatically determined by the assay software. A test result may be negative, positive, or invalid as determined by the Internal Control (IC) RLU and the signal-to-cutoff (S/CO) for the Analyte. A test result may also be invalid due to other parameters (abnormal kinetic curve shape) being outside the normal expected ranges. Invalid test results should be repeated.

APTIMA HPV Assay Result	Criteria
Negative	*Analyte S/CO < 0.50* *Internal Control ≥ IC Cutoff* *Internal Control ≤ 2,000,000 RLU*
Positive	*Analyte S/CO ≥ 0.50* *Internal Control ≤ 2,000,000 RLU* *Analyte ≤ 13,000,000 RLU*
Invalid	*Analyte S/CO < 0.50 and Internal Control < IC Cutoff* *Or Internal Control > 2,000,000 RLU* *Or Analyte > 13,000,000 RLU*

Note: *Negative results are not intended to prevent women from proceeding to colposcopy.*

Note: *Negative results indicate HPV E6/E7 mRNA was not detected.*

Note: *Negative results may occur with HPV E6/E7 mRNA concentrations that are below the pre-set threshold.*

Note: *Positive results indicate the presence of HPV E6/E7 mRNA of any one or more of the high-risk types.*

Note: *Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.*

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Limitations

A. The performance of the APTIMA HPV Assay has not been evaluated for HPV vaccinated individuals.

B. The APTIMA HPV Assay has not been evaluated in cases of suspected sexual abuse.

C. Prevalence of HPV infection in a population may affect performance. Positive predictive values decrease when testing populations with low prevalence or individuals with no risk of infection.

D. PreservCyt liquid cytology specimens containing less than 1 mL after ThinPrep Pap Test slide preparation are considered inadequate for the APTIMA HPV Assay.

E. APTIMA HPV Assay performance has not been evaluated with post-processed PreservCyt specimens using processors other than the ThinPrep 2000 Processor.

F. Test results may be affected by improper specimen collection, storage or specimen processing.

G. A negative APTIMA HPV Assay result does not exclude the possibility of cytologic abnormalities or of future or underlying CIN2, CIN3, or cancer.

H. Personal lubricants that contain Polyquaternium 15 may interfere with the performance of the assay when present at concentrations greater than 0.025% (v/v or w/v) of a test sample.

I. Anti-fungal medications that contain tioconazole may interfere with the performance of the assay when present at concentrations greater than 0.075% (w/v) of a test sample.

J. The APTIMA HPV Assay provides qualitative results. Therefore, a correlation cannot be drawn between the magnitude of a positive assay signal and the expression level of mRNA in a specimen.

K. The APTIMA HPV Assay detects E6/E7 viral messenger RNA (mRNA) of the high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. This test does not detect E6/E7 mRNA of HPV low-risk types (e.g. 6, 11, 42, 43, 44) since there is no clinical utility for testing of low-risk HPV types for cervical cancer screening purposes.[14]

L. Detection of high-risk HPV mRNA is dependent on the number of copies present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection and the presence of interfering substances.

M. Infection with HPV is not an indicator of cytologic HSIL or underlying high-grade CIN, nor does it imply that CIN2, CIN3, or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN2, CIN3, or cancer.

N. The effects of other potential variables such as vaginal discharge, use of tampons, douching, etc. and specimen collection variables have not been evaluated.

O. Use of this product must be limited to personnel trained in the use of the APTIMA HPV Assay.

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P. Cross-contamination of samples can cause false positive results. The sample to sample cross-contamination rate of the APTIMA HPV Assay on the TIGRIS DTS System has been determined in a non-clinical study to be 0.3%.

Q. The APTIMA HPV Assay should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

R. False positive results may occur with this test. In vitro transcripts from low-risk HPV genotypes 26, 67, 70, and 82 exhibited cross-reactivity with the APTIMA HPV Assay.

S. The positive control material is not intended to monitor performance at the assay cutoff.

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Expected Results: Prevalence of High-Risk HPV mRNA

The prevalence of high-risk HPV infection varies widely and is influenced by several factors, for which age is the greatest contributor.[15,16] Many studies have investigated HPV prevalence as determined by the detection of HPV DNA, however few studies report prevalence based on detection of HPV oncogenic mRNA. Women from a variety of clinical sites (n=18) representing a wide geographic distribution and a diverse population (10 states within the United States) were enrolled in a prospective clinical study known as the CLEAR trial. The prevalence of HPV mRNA-positive samples observed in the clinical trial was categorized overall, by age group, and by testing site. Results are shown in Table 1 for the ASC-US and the negative for intraepithelial lesion or malignancy (NILM) populations.

Table 1: High-risk HPV mRNA Prevalence by Age Group, Testing Site, and All Combined

	Positivity Rate % (x/n)	
	ASC-US Population (≥ 21 Years)	**NILM Population (≥ 30 Years)**
All	41.8 (400/958)	5.0 (540/10,871)
Age Group (years)		
21 to 29	60.3 (252/418)	N/A
30 to 39	36.8 (98/266)	6.9 (289/4199)
≥ 40	18.2 (50/274)	3.8 (251/6672)
Testing Site		
1	41.6 (134/322)	4.7 (172/3682)
2	41.4 (150/362)	5.2 (194/3702)
3	42.3 (116/274)	5.0 (174/3487)

N/A = Not Applicable

L8

Assay Performance

APTIMA HPV Assay Clinical Trial Study Design

A prospective, multicenter US clinical study known as the CLEAR trial was conducted to determine the clinical performance of the APTIMA HPV Assay for detection of cervical intraepithelial neoplasia grade 2 or more severe cervical disease (≥CIN2). Women were enrolled into either the ASC-US Study or the NILM Study based on cytology results from routine cervical cancer screening. The ASC-US Study population included women 21 years and older with ASC-US cytology results and the NILM Study population included women 30 years of age and older with NILM cytology results. The NILM Study was designed to support the adjunctive screening claim for women 30 years and older, since women in this age range with cytology results greater than ASC-US should proceed to colposcopy regardless of their HPV status.[14]

Women from 18 clinical sites, primarily obstetrics/gynecology clinics, which covered a wide geographic distribution and a diverse population, were analyzed. Eligible women were assigned to the ASC-US Study or NILM Study based on their referral ThinPrep PreservCyt liquid based cytology specimen. Residual referral specimens were tested with both the APTIMA HPV Assay and an FDA-approved HPV DNA test.

All women in the ASC-US Study were referred to colposcopy, regardless of their HPV test results. An endocervical curettage (ECC) biopsy and cervical punch biopsies (1 biopsy from each of the 4 quadrants) were obtained. If a lesion was visible, a punch biopsy was obtained (directed method; 1 biopsy per lesion) and quadrants without a visible lesion were biopsied at the squamocolumnar junction (random method).

In the NILM Study, women positive with the APTIMA HPV Assay and/or the FDA-approved HPV DNA test, as well as randomly selected women who were negative with both assays, were referred to colposcopy for the baseline evaluation. The randomly selected women who were negative for both assays were included to correct for verification bias with adjusted performance estimates generated using a multiple imputation method. An ECC biopsy was obtained from each woman who attended colposcopy. Punch biopsies were obtained from visible lesions only (directed method; 1 biopsy per lesion). Follow-up of women in the NILM Study who do not have ≥CIN2 is ongoing for 3 years with annual cytology visits. Women with ASC-US or more severe cytology results during the follow-up period are referred to colposcopy using the same biopsy procedure performed for the baseline evaluation.

For both studies, disease status was determined from a consensus histology review panel, which was based on agreement of at least 2 expert pathologists. The expert pathologists were masked to the woman's HPV and cytology status, as well as each other's histology diagnoses. Investigators, clinicians, and women were masked to the HPV test results until after completion of the colposcopy visit, to avoid bias. Clinical performance of the APTIMA HPV Assay was determined for detection of ≥CIN2 and cervical intraepithelial neoplasia grade 3 or more severe cervical disease (≥CIN3). Clinical performance of the FDA-approved HPV DNA test was also determined for direct comparison to the APTIMA HPV Assay results.

ASC-US ≥ 21 Years Population: APTIMA HPV Assay Clinical Performance

In total, there were 1252 women 21 years of age and older with ASC-US cytology results enrolled in the ASC-US Study. Of these, 294 women were withdrawn and 19 had an undetermined disease diagnosis; all were excluded from analysis. The remaining 939 evaluable women were 21 years of age and older with ASC-US cytology results, APTIMA HPV Assay results, and conclusive disease status. Ninety-one (91) women had ≥CIN2 and forty-one (41) had ≥CIN3. Prevalence of ≥CIN2 and ≥CIN3 in evaluable women with ASC-US cytology results were 9.7% and 4.4%, respectively. The results of the APTIMA HPV Assay by the consensus histology review panel diagnoses are presented in Table 2.

Table 2: ASC-US ≥ 21 Years Population: Results of the APTIMA HPV Assay by Consensus Histology Review Panel Diagnosis

APTIMA HPV Assay Result*	HPV DNA Test	Consensus Histology Review Panel Diagnosis						
		Undetermined**	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	Positive	6	170	113	41	32	1	363
Positive	Negative	0	7	0	1	2	0	10
Positive	No Result***	0	14	11	0	2	0	27
Negative	Positive	0	47	13	2	3	0	65
Negative	Negative	10	371	55	6	1	0	443
Negative	No Result***	3	40	7	0	0	0	50
Total		19	649	199	50	40	1****	958

*All samples had final valid results (upon initial testing or after resolution of initial invalids per procedure).

**19 subjects attended the colposcopy visit but a diagnosis could not be determined for the following reasons: < 5 biopsy specimens obtained all with histology results of Normal/CIN1 (n=15), no biopsies collected (n=3), and biopsy slides lost (n=1).

***77 women with APTIMA HPV Assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

****One subject had adenocarcinoma in situ (AIS).

Clinical performance estimates of the APTIMA HPV Assay including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the detection of ≥CIN2 and ≥CIN3 based on evaluating all biopsies and including only directed biopsies are shown in Table 3, as are the estimates for the FDA-approved HPV DNA test.

Table 3: ASC-US ≥ 21 Years Population: Performance of the APTIMA HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 and ≥CIN3

		APTIMA HPV Assay N=939		HPV DNA Test N=865*	
	Performance	Estimate	(95% CI)	Estimate	(95% CI)
≥CIN2	**All Biopsies**				
	Sensitivity (%)	86.8 (79/91)	(78.4, 92.3)	88.8 (79/89)	(80.5, 93.8)
	Specificity (%)	62.9 (533/848)	(59.6, 66.0)	55.8 (433/776)	(52.3, 59.3)
	PPV (%)	20.1 (79/394)	(18.1, 22.0)	18.7 (79/422)	(17.0, 20.4)
	NPV (%)	97.8 (533/545)	(96.5, 98.8)	97.7 (433/443)	(96.2, 98.8)
	Prevalence (%)	9.7 (91/939)		10.3 (89/865)	
	Directed Biopsies**				
	Sensitivity (%)	93.3 (56/60)	(84.1, 97.4)	93.2 (55/59)	(83.8, 97.3)
	Specificity (%)	61.5 (539/876)	(58.3, 64.7)	54.5 (438/804)	(51.0, 57.9)
	PPV (%)	14.2 (56/393)	(12.7, 15.6)	13.1 (55/421)	(11.7, 14.2)
	NPV (%)	99.3 (539/543)	(98.3, 99.8)	99.1 (438/442)	(97.9, 99.7)
	Prevalence (%)	6.4 (60/936)		6.8 (59/863)	
≥CIN3	**All Biopsies**				
	Sensitivity (%)	90.2 (37/41)	(77.5, 96.1)	92.3 (36/39)	(79.7, 97.3)
	Specificity (%)	60.2 (541/898)	(57.0, 63.4)	53.3 (440/826)	(49.9, 56.6)
	PPV (%)	9.4 (37/394)	(8.1, 10.4)	8.5 (36/422)	(7.4, 9.4)
	NPV (%)	99.3 (541/545)	(98.3, 99.8)	99.3 (440/443)	(98.3, 99.8)
	Prevalence (%)	4.4 (41/939)		4.5 (39/865)	
	Directed Biopsies**				
	Sensitivity (%)	93.1 (27/29)	(78.0, 98.1)	96.4 (27/28)	(82.3, 99.4)
	Specificity (%)	59.6 (541/908)	(56.4, 62.7)	52.8 (441/836)	(49.4, 56.1)
	PPV (%)	6.9 (27/394)	(5.8, 7.6)	6.4 (27/422)	(5.5, 7.0)
	NPV (%)	99.6 (541/543)	(98.8, 100)	99.8 (441/442)	(98.9, 100)
	Prevalence (%)	3.1 (29/937)		3.2 (28/864)	

*74 women with APTIMA HPV Assay results did not have HPV DNA Test results primarily due to insufficient volume of the cytology specimen.

**Consensus histology result was derived using only results from directed biopsies. Women with no directed biopsies reflect a normal colposcopy and are included in these analyses as non-diseased (<CIN2 or <CIN3, as appropriate). A consensus was not always reached when only directed biopsies were included.

When evaluating all biopsies, clinical sensitivity estimates of the APTIMA HPV Assay and the FDA-approved HPV DNA test, where both assay results are available for the detection of ≥CIN2 and ≥CIN3, were similar (differences in sensitivity estimates were not statistically significant: sensitivity difference = -2.3% [95% CI: -9.5%, 4.8%]). Clinical specificity estimates of the APTIMA HPV Assay for the detection of of ≥CIN2 and ≥CIN3 were higher than those of the FDA-approved HPV DNA test (differences in specificity estimates were statistically significant). For ≥CIN2, the specificity difference was 6.8% (95% CI: 4.9%, 9.0%). NPVs were similar but for the detection of ≥CIN2, the PPV for the APTIMA HPV Assay was slightly higher than PPV for the FDA-approved HPV DNA test (20.1% vs 18.7%).

Of the 91 ≥CIN2 cases, 60 (65.9%) were identified in directed biopsies and 31 (34.1%) were identified from random and/or ECC biopsies (i.e., not in directed biopsies). These findings are comparable to results from published studies, in which approximately 25% to 40% of ≥CIN2 cases were identified from random and/or ECC biopsy specimens only.[17,18] Using only directed biopsies to determine disease status (assuming women with no directed biopsies had normal histology results because no visible lesions were present), prevalence of ≥CIN2 and ≥CIN3 in the study were 6.4% and 3.1%, respectively. The clinical sensitivity estimates for the detection of ≥CIN2 and ≥CIN3 were higher for both tests using directed biopsies only than estimates calculated using all biopsies. For both assays, clinical specificity using only directed biopsies was similar to the specificity obtained with all biopsies included. Accordingly, when using only directed biopsies, the APTIMA HPV Assay specificity was significantly higher than that of the FDA-approved HPV DNA test.

Clinical performance estimates of the APTIMA HPV Assay and the FDA-approved HPV DNA test are shown by age group in Table 4 and Table 5 (≥CIN2 and ≥CIN3, respectively, based on evaluating all biopsies).

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Table 4: ASC-US ≥ 21 Years Population: Performance of the APTIMA HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 by Age Group

		APTIMA HPV Assay N=939		HPV DNA test N=865*	
	Performance	Estimate	(95% CI)	Estimate	(95% CI)
21 to 29 Years		N=415		N=389	
	Sensitivity (%)	90.2 (55/61)	(80.2, 95.4)	94.9 (56/59)	(86.1, 98.3)
	Specificity (%)	44.9 (159/354)	(39.8, 50.1)	35.5 (117/330)	(30.5, 40.8)
	PPV (%)	22.0 (55/250)	(19.6, 24.2)	20.8 (56/269)	(19.0, 22.5)
	NPV (%)	96.4 (159/165)	(93.0, 98.5)	97.5 (117/120)	(93.6, 99.4)
	Prevalence (%)	14.7 (61/415)		15.2 (59/389)	
30 to 39 Years		N=262		N=239	
	Sensitivity (%)	90.0 (18/20)	(69.9, 97.2)	80.0 (16/20)	(58.4, 91.9)
	Specificity (%)	68.2 (165/242)	(62.1, 73.7)	61.6 (135/219)	(55.1, 67.8)
	PPV (%)	18.9 (18/95)	(14.7, 22.7)	16.0 (16/100)	(11.8, 19.6)
	NPV (%)	98.8 (165/167)	(96.5, 99.8)	97.1 (135/139)	(94.1, 99.1)
	Prevalence (%)	7.6 (20/262)		8.4 (20/239)	
≥ 40 Years		N=262		N=237	
	Sensitivity (%)	60.0 (6/10)	(31.3, 83.2)	70.0 (7/10)	(39.7, 89.2)
	Specificity (%)	82.9 (209/252)	(77.8, 87.1)	79.7 (181/227)	(74.0, 84.4)
	PPV (%)	12.2 (6/49)	(5.8, 18.4)	13.2 (7/53)	(6.9, 18.7)
	NPV (%)	98.1 (209/213)	(96.6, 99.4)	98.4 (181/184)	(96.6, 99.6)
	Prevalence (%)	3.8 (10/262)		4.2 (10/237)	

*74 women with APTIMA HPV Assay results did not have HPV DNA Test results primarily due to insufficient volume of the cytology specimen.

Table 5: ASC-US ≥ 21 Years Population: Performance of the APTIMA HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN3 by Age Group

	Performance	APTIMA HPV Assay N=939 Estimate	APTIMA HPV Assay (95% CI)	HPV DNA test N=865* Estimate	HPV DNA test (95% CI)
21 to 29 Years		N=415		N=389	
	Sensitivity (%)	96.3 (26/27)	(81.7, 99.3)	100 (25/25)	(86.7, 100)
	Specificity (%)	42.3 (164/388)	(37.5, 47.2)	33.0 (120/364)	(28.3, 38.0)
	PPV (%)	10.4 (26/250)	(8.9, 11.4)	9.3 (25/269)	(8.2, 10.0)
	NPV (%)	99.4 (164/165)	(97.2, 100)	100 (120/120)	(97.5, 100)
	Prevalence (%)	6.5 (27/415)		6.4 (25/389)	
30 to 39 Years		N=262		N=239	
	Sensitivity (%)	88.9 (8/9)	(56.5, 98.0)	77.8 (7/9)	(45.3, 93.7)
	Specificity (%)	65.6 (166/253)	(59.6, 71.2)	59.6 (137/230)	(53.1, 65.7)
	PPV (%)	8.4 (8/95)	(5.2, 10.4)	7.0 (7/100)	(3.9, 9.1)
	NPV (%)	99.4 (166/167)	(97.6, 100)	98.6 (137/139)	(96.4, 99.8)
	Prevalence (%)	3.4 (9/262)		3.8 (9/239)	
≥ 40 Years		N=262		N=237	
	Sensitivity (%)	60.0 (3/5)	(23.1, 88.2)	80.0 (4/5)	(37.6, 96.4)
	Specificity (%)	82.1 (211/257)	(77.0, 86.3)	78.9 (183/232)	(73.2, 83.6)
	PPV (%)	6.1 (3/49)	(1.6, 10.2)	7.5 (4/53)	(2.9, 10.7)
	NPV (%)	99.1 (211/213)	(98.0, 99.9)	99.5 (183/184)	(98.2, 100)
	Prevalence (%)	1.9 (5/262)		2.1 (5/237)	

*74 women with APTIMA HPV Assay results did not have HPV DNA Test results primarily due to insufficient volume of the cytology specimen.

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The absolute risk of disease (≥CIN2 and ≥CIN3, based on evaluating all biopsies) by APTIMA HPV Assay result and the relative risk of disease for positive versus negative APTIMA HPV Assay results are shown in Table 6, as are the estimates for the FDA-approved HPV DNA test. The relative risk of ≥CIN2 was 9.1 (95% CI: 5.0, 16.5), indicating that a woman who was APTIMA HPV Assay positive was 9.1 times as likely to have ≥CIN2 than a woman who was APTIMA HPV Assay negative. The relative risk of ≥CIN3 was 12.8 (95% CI: 4.6, 35.6).

Table 6: ASC-US ≥ 21 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV Assay and an FDA-approved HPV DNA Test

		APTIMA HPV Assay N=939		**HPV DNA test N=865***	
	Assay Result	**Absolute Risk (95% CI)**	**Relative Risk (95% CI)**	**Absolute Risk (95% CI)**	**Relative Risk (95% CI)**
≥CIN2	**Positive**	20.1 (79/394) (18.1, 22.0)	9.1 (5.0, 16.5)	18.7 (79/422) (17.0, 20.4)	8.3 (4.4, 15.8)
	Negative	2.2 (12/545) (1.2, 3.5)		2.3 (10/443) (1.2, 3.8)	
	Prevalence (%)	9.7 (91/939)		10.3 (89/865)	
≥CIN3	**Positive**	9.4 (37/394) (8.1, 10.4)	12.8 (4.6, 35.6)	8.5 (36/422) (7.4, 9.4)	12.6 (3.9, 40.6)
	Negative	0.7 (4/545) (0.2, 1.7)		0.7 (3/443) (0.2, 1.7)	
	Prevalence (%)	4.4 (41/939)		4.5 (39/865)	

*74 women with APTIMA HPV Assay results did not have HPV DNA Test results primarily due to insufficient volume of the cytology specimen.

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Absolute and relative risk estimates of disease (≥CIN2 and ≥CIN3, based on evaluating all biopsies) for the APTIMA HPV Assay and the FDA-approved HPV DNA test are shown by age group in Table 7.

Table 7: ASC-US ≥ 21 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV Assay and an FDA-approved HPV DNA Test by Age Group

			APTIMA HPV Assay N=939		**HPV DNA test N=865***	
	Age	**Assay Result**	**Absolute Risk (95% CI)**	**Relative Risk (95% CI)**	**Absolute Risk (95% CI)**	**Relative Risk (95% CI)**
≥CIN2	21 to 29 Years		N=415		N=389	
		Positive	22.0 (55/250) (19.6, 24.2)	6.1 (2.7, 13.7)	20.8 (56/269) (19.0, 22.5)	8.3 (2.7, 26.1)
		Negative	3.6 (6/165) (1.5, 7.0)		2.5 (3/120) (0.6, 6.4)	
		Prevalence (%)	14.7 (61/415)		15.2 (59/389)	
	30 to 39 Years		N=262		N=239	
		Positive	18.9 (18/95) (14.7, 22.7)	15.8 (3.8, 66.7)	16.0 (16/100) (11.8, 19.6)	5.6 (1.9, 16.1)
		Negative	1.2 (2/167) (0.2, 3.5)		2.9 (4/139) (0.9, 5.9)	
		Prevalence (%)	7.6 (20/262)		8.4 (20/239)	
	≥ 40 Years		N=262		N=237	
		Positive	12.2 (6/49) (5.8, 18.4)	6.5 (1.9, 22.2)	13.2 (7/53) (6.9, 18.7)	8.1 (2.2, 30.2)
		Negative	1.9 (4/213) (0.6, 3.4)		1.6 (3/184) (0.4, 3.4)	
		Prevalence (%)	3.8 (10/262)		4.2 (10/237)	
≥CIN3	21 to 29 Years		N=415		N=389	
		Positive	10.4 (26/250) (8.9, 11.4)	17.2 (2.4, 125)	9.3 (25/269) (8.2, 10.0)	Not Calculable
		Negative	0.6 (1/165) (0.0, 2.8)		0.0 (0/120) (0.0, 2.5)	
		Prevalence (%)	6.5 (27/415)		6.4 (25/389)	
	30 to 39 Years		N=262		N=239	
		Positive	8.4 (8/95) (5.2, 10.4)	14.1 (1.8, 111)	7.0 (7/100) (3.9, 9.1)	4.9 (1.0, 22.9)
		Negative	0.6 (1/167) (0.0, 2.4)		1.4 (2/139) (0.2, 3.6)	
		Prevalence (%)	3.4 (9/262)		3.8 (9/239)	
	≥ 40 Years		N=262		N=237	
		Positive	6.1 (3/49) (1.6, 10.2)	6.5 (1.1, 38.0)	7.5 (4/53) (2.9, 10.7)	13.9 (1.6, 122)
		Negative	0.9 (2/213) (0.1, 2.0)		0.5 (1/184) (0.0, 1.8)	
		Prevalence (%)	1.9 (5/262)		2.1 (5/237)	

*74 women with APTIMA HPV Assay results did not have HPV DNA Test results primarily due to insufficient volume of the cytology specimen.

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NILM ≥ 30 Years Population (NILM Study): APTIMA HPV Assay Clinical Performance

In total, there were 11,644 women with NILM cytology results enrolled in the NILM Study. Of these, 773 women were withdrawn and excluded from analysis. The remaining 10,871 evaluable women were 30 years of age and older with NILM cytology results and APTIMA HPV Assay results. Of the 540 women with positive APTIMA HPV Assay results, 335 attended colposcopy. Of the 10,331 women with negative APTIMA HPV Assay results, 530 attended colposcopy. Twenty (20) women had ≥CIN2 and eleven (11) had ≥CIN3; 799 women had Normal/CIN1 histology; 46 women had undetermined disease status. The results of the APTIMA HPV Assay by the consensus histology review panel diagnosis are presented in Table 8.

Table 8: NILM ≥ 30 Years Population: Results of the APTIMA HPV Assay and Consensus Histology Review Panel Diagnosis

APTIMA HPV Assay Result*	HPV DNA Test	Consensus Histology Review Panel Diagnosis						
		Undetermined	Normal	CIN1	CIN2	CIN3	Cancer	Total
Positive	Positive	11	212	11	4	7	2	247
Positive	Negative	7	59	0	1	0	1	68
Positive	No Result**	3	16	1	0	0	0	20
Negative	Positive	10	170	8	2	1	0	191
Negative	Negative	15	313	9	1	0	0	338
Negative	No Result**	0	0	0	1	0	0	1
Total		46	770	29	9	8	3***	865

*All samples had final valid results (upon initial testing or after resolution of initial invalids per procedure).

**21 women with APTIMA HPV Assay results did not have HPV DNA test results primarily due to insufficient volume of the cytology specimen.

***Three women had adenocarcinoma in situ (AIS).

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In total, 10,052 women had unverified (including undetermined) disease status (Table 9). Because only randomly selected women with negative results for both the APTIMA HPV Assay and the FDA-approved HPV DNA test were referred to colposcopy, the proportion of women with unverified disease status was high in this group (96.6%). To adjust for this verification bias, a multiple imputation method was used to estimate the number of women with disease that would have been identified if all women had undergone colposcopy. Both verification-bias adjusted performance estimates and unadjusted performance estimates based on the 819 women with verified disease status are presented.

Table 9: NILM ≥ 30 Years Population: Classification of Evaluable NILM Women by APTIMA HPV Assay and HPV DNA Test Results, Disease Status (≥CIN2 and ≥CIN3), and Disease Verification Status

APTIMA HPV Assay Result*	HPV DNA Test	Total Women	Verified Disease Status: ≥CIN2		Verified Disease Status: ≥CIN3		Unverified Disease Status
			Diseased Women (≥CIN2)	Non-Diseased Women (<CIN2)	Diseased Women (≥CIN3)	Non-Diseased Women (<CIN3)	Women with Unknown Disease Status (% Unknown)
Positive	Positive	360	13	223	9	227	124 (34.4%)
Positive	Negative	150	2	59	1	60	89 (59.3%)
Positive	No Result**	30	0	17	0	17	13 (43.3%)
Negative	Positive	306	3	178	1	180	125 (40.8%)
Negative	Negative	9420	1	322	0	323	9097 (96.6%)
Negative	No Result**	605	1	0	0	1	604 (99.8%)
Total		10,871	20	799	11	808	10,052 (92.5%)

*All samples had final results (upon initial testing or after resolution of initial invalids per procedure).

**635 women with APTIMA HPV Assay results did not have HPV DNA Test results primarily due to insufficient volume of the cytology specimen.

The adjusted prevalence of ≥CIN2 and ≥CIN3 in women with NILM cytology results were 0.9% and 0.4%, respectively. The adjusted absolute and relative risk estimates for detection of ≥CIN2 and ≥CIN3 are shown in Table 10. The adjusted relative risk of ≥CIN2 was 8.1 (95% CI: 2.3, 28.1), indicating that a woman who was APTIMA HPV Assay positive is 8.1 times as likely to have ≥CIN2 than a woman who is APTIMA HPV Assay negative. The adjusted relative risk of ≥CIN3 was 34.5 (95% CI: 2.7, 443.3). The unadjusted absolute and relative risk estimates for detection of ≥CIN2 and ≥CIN3 are shown overall in Table 11 and by age group in Table 12.

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Table 10: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV Assay and an FDA-approved HPV DNA Test (Verification-Bias Adjusted Estimates)

Assay Result		APTIMA HPV Assay Absolute Risk (95% CI)	APTIMA HPV Assay Relative Risk (95% CI)	HPV DNA test Absolute Risk (95% CI)	HPV DNA test Relative Risk (95% CI)
≥CIN2	Positive	4.7 (2.9, 7.6)	8.1 (2.3, 28.1)	3.7 (2.3, 6.0)	7.3 (1.6, 33.4)
	Negative	0.6 (0.2, 1.9)		0.5 (0.1, 2.1)	
	Prevalence (%)	0.9		0.9	
≥CIN3	Positive	3.3 (1.4, 7.6)	34.5 (2.7, 443.3)	2.3 (1.3, 4.1)	21.0 (1.0, 423.4)
	Negative	0.1 (0.0, 1.6)		0.1 (0.0, 2.4)	
	Prevalence (%)	0.4		0.4	

Table 11: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV Assay and an FDA-approved HPV DNA Test (Unadjusted Estimates)

Assay Result		APTIMA HPV Assay N=819 Absolute Risk (95% CI)	APTIMA HPV Assay N=819 Relative Risk (95% CI)	HPV DNA test N=801* Absolute Risk (95% CI)	HPV DNA test N=801* Relative Risk (95% CI)
≥CIN2	Positive	4.8 (15/314) (3.4, 5.8)	4.8 (1.8, 13.1)	3.8 (16/417) (2.9, 4.4)	4.9 (1.4, 16.7)
	Negative	1.0 (5/505) (0.4, 1.9)		0.8 (3/384) (0.2, 1.9)	
	Prevalence (%)	2.4 (20/819)		2.4 (19/801)	
≥CIN3	Positive	3.2 (10/314) (2.2, 3.7)	16.1 (2.1, 125)	2.4 (10/417) (1.6, 2.7)	9.2 (1.2, 71.6)
	Negative	0.2 (1/505) (0.0, 0.9)		0.3 (1/384) (0.0, 1.1)	
	Prevalence (%)	1.3 (11/819)		1.4 (11/801)	

*18 women with APTIMA HPV Assay results did not have HPV DNA Test results primarily due to insufficient volume of the cytology specimen.

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Table 12: NILM ≥ 30 Years Population: Absolute and Relative Risks of ≥CIN2 and ≥CIN3 for Results of the APTIMA HPV Assay and an FDA-approved HPV DNA Test by Age Group (Unadjusted Estimates)

			APTIMA HPV Assay N=819		HPV DNA test N=801*	
	Age	Assay Result	Absolute Risk (95% CI)	Relative Risk (95% CI)	Absolute Risk (95% CI)	Relative Risk (95% CI)
≥CIN2	30 to 39 Years		N=384		N=377	
		Positive	4.8 (8/167) (2.1, 9.2)	10.4 (1.3, 82.3)	3.2 (7/216) (1.3, 6.6)	2.6 (0.5, 12.4)
		Negative	0.5 (1/217) (0.0, 2.5)		1.2 (2/161) (0.2, 4.4)	
		Prevalence (%)	2.3 (9/384)		2.4 (9/377)	
	≥ 40 Years		N=435		N=424	
		Positive	4.8 (7/147) (1.9, 9.6)	3.4 (1.0, 11.5)	4.5 (9/201) (2.1, 8.3)	10.0 (1.3, 78.1)
		Negative	1.4 (4/288) (0.4, 3.5)		0.4 (1/223) (0.0, 2.5)	
		Prevalence (%)	2.5 (11/435)		2.4 (10/424)	
≥CIN3	30 to 39 Years		N=384		N=377	
		Positive	3.0 (5/167) (1.0, 6.8)	6.5 (0.8, 55.1)	2.3 (5/216) (0.8, 5.3)	3.7 (0.4, 31.6)
		Negative	0.5 (1/217) (0.0, 2.5)		0.6 (1/161) (0.0, 3.4)	
		Prevalence (%)	1.6 (6/384)		1.6 (6/377)	
	≥ 40 Years		N=435		N=424	
		Positive	3.4 (5/147) (1.1, 7.8)	Not Calculable	2.5 (5/201) (0.8, 5.7)	Not Calculable
		Negative	0.0 (0/288) (0.0, 1.3)		0.0 (0/223) (0.0, 1.6)	
		Prevalence (%)	1.1 (5/435)		1.2 (5/424)	

*18 women with APTIMA HPV Assay results did not have HPV DNA Test results primarily due to insufficient volume of the cytology specimen.

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Adjusted clinical performance estimates of the APTIMA HPV Assay including sensitivity, specificity, PPV, and NPV for the detection of ≥CIN2 and ≥CIN3 are shown in Table 13, as are the estimates for the FDA-approved HPV DNA test. Unadjusted clinical performance estimates are shown in Table 14. The APTIMA HPV Assay and the FDA-approved HPV DNA test had similar sensitivity, whereas specificity was significantly higher for the APTIMA HPV Assay (non-overlapping 95% CIs). Predictive value estimates of the APTIMA HPV Assay were clinically relevant and similar to the estimates for the FDA-approved HPV DNA test. NPVs were similar but for the detection of ≥CIN2, the PPV for the APTIMA HPV Assay was slightly higher than PPV for the FDA-approved HPV DNA test (4.7% vs 3.7%).

Table 13: NILM ≥ 30 Years Population: Performance of the APTIMA HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 and ≥CIN3 (Verification-Bias Adjusted Estimates)

	Performance	APTIMA HPV Assay		HPV DNA test	
		Estimate	(95% CI)	Estimate	(95% CI)
≥CIN2	Sensitivity (%)	31.0	(5.9, 56.1)	35.4	(3.8, 66.9)
	Specificity (%)	95.2	(94.8, 95.6)	93.7	(93.2, 94.2)
	PPV (%)	4.7	(2.9, 7.6)	3.7	(2.3, 6.0)
	NPV (%)	99.4	(98.1, 99.8)	99.5	(97.9, 99.9)
	Prevalence (%)	0.9		0.9	
≥CIN3	Sensitivity (%)	61.5	(14.0, 100)	56.4	(0.4, 100)
	Specificity (%)	95.2	(94.8, 95.6)	93.6	(93.1, 94.1)
	PPV (%)	3.3	(1.4, 7.6)	2.3	(1.3, 4.1)
	NPV (%)	99.9	(98.4, 100)	99.9	(97.6, 100)
	Prevalence (%)	0.4		0.4	

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Table 14: NILM ≥ 30 Years Population: Performance of the APTIMA HPV Assay and an FDA-approved HPV DNA Test for Detection of ≥CIN2 and ≥CIN3 (Unadjusted Estimates)

	Performance	APTIMA HPV Assay N=819		HPV DNA test N=801*	
		Estimate	**(95% CI)**	**Estimate**	**(95% CI)**
≥CIN2	**Sensitivity (%)**	75.0 (15/20)	(53.1, 88.8)	84.2 (16/19)	(62.4, 94.5)
	Specificity (%)	62.6 (500/799)	(59.2, 65.9)	48.7 (381/782)	(45.2, 52.2)
	PPV (%)	4.8 (15/314)	(3.4, 5.8)	3.8 (16/417)	(2.9, 4.4)
	NPV (%)	99.0 (500/505)	(98.1, 99.6)	99.2 (381/384)	(98.1, 99.8)
	Prevalence (%)	2.4 (20/819)		2.4 (19/801)	
≥CIN3	**Sensitivity (%)**	90.9 (10/11)	(62.3, 98.4)	90.9 (10/11)	(62.3, 98.4)
	Specificity (%)	62.4 (504/808)	(59.0, 65.7)	48.5 (383/790)	(45.0, 52.0)
	PPV (%)	3.2 (10/314)	(2.2, 3.7)	2.4 (10/417)	(1.6, 2.7)
	NPV (%)	99.8 (504/505)	(99.1, 100)	99.7 (383/384)	(98.9, 100)
	Prevalence (%)	1.3 (11/819)		1.4 (11/801)	

*18 women with APTIMA HPV Assay results did not have HPV DNA Test results primarily due to insufficient volume of the cytology specimen.

While establishing clinical sensitivity and specificity requires completion of the 3-year follow-up, direct comparison of the APTIMA HPV Assay and the FDA-approved HPV DNA test demonstrates similar sensitivity and statistically significant improved specificity of the APTIMA HPV Assay over the FDA-approved HPV DNA test for detection of ≥CIN2 as shown by the ratios of true positive and false positive rates (Table 15 and Table 16, respectively).

Table 15: NILM ≥ 30 Years Population: Ratio of True Positive Rates (APTIMA HPV Assay/ FDA-approved HPV DNA Test) for Women with ≥CIN2 (Unadjusted Estimates)

		HPV DNA Test		
		Positive	**Negative**	**Total**
APTIMA HPV Assay	**Positive**	13	2	15 (78.9%)
	Negative	3	1	4
	Total	16 (84.2%)	3	19
Ratio of True Positive Rates = 0.94 (15/16) (95% CI: 0.67, 1.20)				

Table 16: NILM ≥ 30 Years Population: Ratio of False Positive Rates (APTIMA HPV Assay/ FDA-approved HPV DNA Test) for Women with <CIN2 (Unadjusted Estimates)

		HPV DNA Test		
		Positive	**Negative**	**Total**
APTIMA HPV Assay	**Positive**	223	59	282 (36.1%)
	Negative	178	322	500
	Total	401 (51.3%)	381	782
Ratio of False Positive Rates = 0.70 (282/401) (95% CI: 0.64, 0.77)				

APTIMA HPV Assay Agreement with a Composite Comparator

The analytical performance of the APTIMA HPV Assay was assessed against a composite comparator consisting of an FDA-approved HPV DNA test and a validated reverse transcription-polymerase chain reaction (RT-PCR) sequencing test specific for E6/E7 mRNA from the same 14 HR HPV types detected by the APTIMA HPV Assay. Sequencing was performed by an external commercial laboratory.

Cervical specimens collected from the ASC-US and NILM populations of the CLEAR trial were tested by the comparator assays and compared to the APTIMA HPV Assay results. In total, 434 samples were tested, with 217 from each of the ASC-US and NILM populations.

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Women selected included approximately 150 randomly selected women with positive APTIMA HPV Assay results and <CIN2 disease, approximately 200 randomly selected women with negative APTIMA HPV Assay results and <CIN2 disease and all women identified with ≥CIN2 disease by consensus histology review at the time of sample selection (when about 80% of the trial enrollment was completed).

For the composite comparator analysis, samples were classified positive if the HPV DNA test and the E6/E7 RT-PCR sequencing test were both positive; negative if the HPV DNA test and the E6/E7 RT-PCR sequencing test were both negative; and indeterminate if the tests were discordant, or if one or both tests returned an invalid or indeterminate result.

Positive and negative percent agreements and associated 95% confidence intervals were calculated. Indeterminate results were not included in the agreement calculations. Results are presented for the ASC-US population (≥ 21 years) and the NILM population (≥ 30 years) in Table 17a and Table 17b, respectively.

Table 17a: ASC-US ≥ 21 Years Population: APTIMA HPV Assay Agreement Results with a Composite Comparator (n=217)

		Composite Comparator			
		Positive	**Negative**	**Indeterminate**	**Total**
APTIMA HPV Assay	**Positive**	89	0	27	116
	Negative	2	86	13	101
	Total	91	86	40	217
Positive Percent Agreement: 97.8% (89/91) (95% CI: 92.3, 99.4)					
Negative Percent Agreement: 100.0% (86/86) (95% CI: 95.7, 100)					

Table 17b: NILM ≥ 30 Years Population: APTIMA HPV Assay Agreement Results with a Composite Comparator (n=217)

		Composite Comparator			
		Positive	**Negative**	**Indeterminate**	**Total**
APTIMA HPV Assay	**Positive**	55	15	46	116
	Negative	4	63	34	101
	Total	59	78	80	217
Positive Percent Agreement: 93.2% (55/59) (95% CI: 83.8, 97.3)					
Negative Percent Agreement: 80.8% (63/78) (95% CI: 70.7, 88.0)					

Clinical Cutoff Determination for the APTIMA HPV Assay

The clinical cutoff for detecting high-grade cervical disease (≥CIN2) for the APTIMA HPV Assay was established based on the evaluation of approximately 1000 women with ASC-US cytology results enrolled into the ASC-US Study. The method[19] used for selection of the cutoff was chosen to achieve the maximum sensitivity for detecting ≥CIN2 while maintaining a clinically acceptable level of specificity in the ASC-US population. Based on the method described above, the cutoff for the APTIMA HPV Assay was set at 0.50 S/CO.

Limit of Detection at the Clinical Cutoff

The Limit of Detection (LOD) at the clinical cutoff is the concentration of HPV RNA that gives a positive result (above the clinical cutoff) 95% of the time. The LoD of the APTIMA HPV Assay was determined by testing individual negative clinical PreservCyt liquid cytology specimens spiked with HPV in vitro transcripts or infected cells at various concentrations. Thirty replicates of each copy level were tested with each of three reagent lots for a total of 90 replicates. Testing was performed over 14 days, with 1 to 14 runs performed per day and 30 replicates of a given genotype tested in each run. The 95% detection limit was calculated from Probit regression analysis of the positivity results for each dilutional panel.

The Probit analysis results, Table 18, show that HPV 16, 18, 31, 33, 35, 39, 45, 58, 59, 66, and 68 had 95% detection limits less than 100 copies/reaction; and types 51, 52, and 56 had 95% detection limits between 100 and 300 copies/reaction. The four cell lines tested had 95% detection limits less than 1 cell/reaction. Each target analyte was diluted in STM prior to adding PreservCyt media at a 1:2.9 ratio for these LOD panel specimens. Therefore, the results of this study do not reflect any effects of sampling from a heterogenous non-lysed whole cell suspension prior to PreservCyt specimen transfer to STM.

Table 18: Limit of Detection at Clinical Cutoff of the APTIMA HPV Assay

Target	Limit of Detection* (95% CI)
HPV 16	27.1 (21.7 - 36.1)
HPV 18	54.1 (42.9 - 73.2)
HPV 31	11.5 (9.2 - 15.6)
HPV 33	34.4 (26.9 - 47.4)
HPV 35	31.9 (23.6 - 47.3)
HPV 39	20.5 (16.2 - 28.0)
HPV 45	26.3 (21.1 - 34.9)
HPV 51	132.9 (116.7 - 159.6)
HPV 52	240.2 (191.4 - 320.3)
HPV 56	105.5 (88.0 - 133.2)
HPV 58	71.4 (55.9 - 97.3)
HPV 59	62.3 (47.7 - 89.7)
HPV 66	208.0 (168.4 - 270.0)
HPV 68	47.1 (35.9 - 67.9)
SiHa	0.288 (0.223 - 0.401)
HeLa	0.029 (0.024 - 0.036)
ME180	0.0012 (0.0009 - 0.0018)
MS751	0.018 (0.015 - 0.024)

* Copies per reaction for in vitro transcripts and cells per reaction for cell lines

Assay Precision

APTIMA HPV Assay precision was evaluated in two studies using the same 20-member panel. Study 1 was conducted at 3 external testing sites to determine assay reproducibility. Study 2 was conducted in-house to measure assay repeatability. The panel included 10 HPV-positive members with concentrations at or above the limit of detection of the assay (expected positivity: ≥ 95%), 4 HPV-positive members with concentrations below the limit of detection of the assay (expected positivity: >0% to <25%), and 6 HPV-negative members. HPV-positive panel members were prepared by spiking in vitro RNA transcripts (IVT) into specimen transport medium (STM) or HPV-infected cultured cells (SiHa, HeLa, ME180 and MS751; ATCC, Manassas, Virginia) into PreservCyt Solution. HPV-negative panel members were prepared with STM or pooled residual PreservCyt Solution specimens.

In Study 1, 2 operators at each of the 3 testing sites (1 instrument per site) performed 1 APTIMA HPV Assay worklist per day over 3 days for each of 3 reagent lots. Each worklist contained 3 replicates of each of the reproducibility panel members. One hundred sixty-two (162) individual sample tubes were tested for each panel member (3 sites x 1 instrument x 2 operators x 3 lots x 3 worklists x 3 replicates). In Study 2, testing was conducted in-house over 20 days with a total of 162 reactions tested for each panel member (1 site x 3 instruments x 3 operators x 3 lots x 2 worklists x 3 replicates).

The panel members are described in Table 19a (panel members with expected positive results) and Table 19b (panel members with expected negative results), along with a

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summary of the agreement with expected results and analyte S/CO values at the 2.5th, 50th and 97.5th percentiles of the S/CO distribution. The analyte S/CO variability for the panel members with expected positive results is shown in Table 20 for Study 1 and Table 21 for Study 2.

Positive agreement for the HPV-positive panel members with concentrations at or above the limit of detection of the assay ranged from 95.1% to 100% in Study 1 and from 93.2% to 100% in Study 2 for 9 of the 10 panel members. The remaining HPV-positive panel member yielded 77.2% agreement in Study 1 and 79.0% agreement in Study 2, which was lower than expected, but was consistent between the 2 studies. Negative agreement for the HPV-high negative panel members with concentrations below the limit of detection of the assay ranged from 78.8% to 93.8% in Study 1 and from 82.1% to 95.7% in Study 2. Agreement with expected results for the HPV-negative panel members ranged from 96.9% to 100% in Study 1 and from 96.3% to 100% in Study 2.

Table 19a: APTIMA HPV Assay Reproducibility Study 1 and 2: Panel Description, Positive Agreement, and Percentile Distribution of Analyte S/CO Values for Panel Members with Expected Positive Results

Panel Description (copies or cells/reaction)	Study 1 (3 testing sites)				Study 2 (1 testing site)			
	% positive agreement (95% CI)	Analyte S/CO Percentile			% positive agreement (95% CI)	Analyte S/CO Percentile		
		2.5th	50th	97.5th		2.5th	50th	97.5th
HPV 16 & HPV 18 IVT (100 copies)	100 (161/161) (97.7, 100)	20.7	23.5	26.3	100 (162/162) (97.7, 100)	20.1	23.2	26.3
SiHa cells (3 cells) & HeLa cells (7.5 cells)	100 (162/162) (97.7, 100)	11.0	15.3	28.0	100 (162/162) (97.7, 100)	12.5	16.5	28.0
HPV 18 IVT (100 copies)	100 (162/162) (97.7, 100)	8.5	11.8	13.9	100 (160/160) (97.7, 100)	9.0	11.9	14.7
HPV 16 IVT (100 copies)	100 (162/162) (97.7, 100)	9.9	10.8	11.6	100 (162/162) (97.7, 100)	9.3	10.9	11.7
MS751 cells (1 cell)	99.4 (161/162) (96.6, 99.9)	6.1	13.9	16.0	96.9 (157/162) (93.0, 98.7)	0	14.5	16.6
ME180 cells (0.3 cells)	95.1 (154/162) (90.6, 97.5)	0	7.3	9.3	93.2 (151/162) (88.3, 96.2)	0	6.5	9.4
HPV 18 IVT (30 copies)	99.4 (161/162) (96.6, 99.9)	3.0	9.3	13.3	100 (162/162) (97.7, 100)	4.3	8.7	13.6
HPV 16 IVT (30 copies)	100 (162/162) (97.7, 100)	8.2	10.9	11.8	97.5 (158/162) (93.8, 99.0)	4.3	11.0	11.9
HeLa cells (2.5 cells)	100 (162/162) (97.7, 100)	6.6	12.9	15.8	95.6 (152/159) (91.2, 97.9)	0	12.9	16.5
SiHa cells (1 cell)*	77.2 (125/162) (70.1, 83.0)	0	10.5	12.1	79.0 (128/162) (72.1, 84.6)	0	10.5	11.8

IVT = in vitro transcript. IVT was spiked into STM and cells were spiked into PreservCyt Solution.

*Expected % positive agreement ~95%; observed lower possibly due to manufacturing variability of the panel member.

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Table 19b: APTIMA HPV Assay Reproducibility Study 1 and 2: Panel Description, Negative Agreement, and Percentile Distribution of Analyte S/CO Values for Panel Members with Expected Negative Results

Panel Description (copies or cells/reaction)	Study 1 (3 testing sites)				Study 2 (1 testing site)			
	% negative agreement (95% CI)	Analyte S/CO Percentile			% negative agreement (95% CI)	Analyte S/CO Percentile		
		2.5th	50th	97.5th		2.5th	50th	97.5th
HPV 18 IVT (1 copy)*	78.8 (126/160) (71.8, 84.4)	0	0	4.6	83.3 (135/162) (76.8, 88.3)	0	0	5.2
HPV 16 IVT (1 copy)*	80.9 (131/162) (74.1, 86.2)	0	0	10.7	88.3 (143/162) (82.4, 92.4)	0	0	11.3
HeLa cells (0.05 cells)*	79.0 (128/162) (72.1, 84.6)	0	0	10.4	82.1 (133/162) (75.5, 87.2)	0	0	11.6
SiHa cells (0.03 cells)*	93.8 (152/162) (89.0, 96.6)	0	0	10.7	95.7 (155/162) (91.4, 97.9)	0	0	6.9
STM Lot 1	100 (162/162) (97.7, 100)	0	0	0.1	100 (162/162) (97.7, 100)	0	0	0
STM Lot 2	99.4 (160/161) (96.6, 99.9)	0	0	0.2	100 (162/162) (97.7, 100)	0	0	0
STM Lot 3	99.4 (161/162) (96.6, 99.9)	0	0	0.1	99.4 (161/162) (96.6, 99.9)	0	0	0
ThinPrep Pool 1	97.5 (158/162) (93.8, 99.0)	0	0	0.3	97.5 (158/162) (93.8, 99.0)	0	0	0.3
ThinPrep Pool 2	96.9 (157/162) (93.0, 98.7)	0	0	0.7	96.3 (156/162) (92.2, 98.3)	0	0	1.6
ThinPrep Pool 3	100 (162/162) (97.7, 100)	0	0	0.2	99.4 (161/162) (96.6, 99.9)	0	0	0

STM = specimen transport medium; IVT = in vitro transcript. IVT was spiked into STM and cells were spiked into PreservCyt Solution.

* Expected % negative agreement > 75% and < 100%.

Table 20: APTIMA HPV Assay Reproducibility Study 1: Signal Variability for Panel Members With Expected Positive Results

Panel Description (copies or cells/reaction)	n	Mean S/CO	Between Sites		Between Operators		Between Lots		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPV 16 & HPV 18 IVT (100 copies)	161^	23.4	0.1	0.4	0.1	0.4	0.9	4.0	0	0	1.6	7.0	1.9	8.1
SiHa cells (3 cells) & HeLa cells (7.5 cells)	162	17.9	0	0	1.4	8.1	0	0	0.6	3.1	5.1	28.6	5.3	29.9
HPV 18 IVT (100 copies)	162	11.8	0	0	0	0	0.8	6.4	0.1	0.9	1.2	10.1	1.4	12.0
HPV 16 IVT (100 copies)	162	10.8	0.2	1.5	0	0	0.1	1.1	0.3	2.6	0.3	3.1	0.5	4.5
MS751 cells (1 cell)	162	13.3	0.3	2.1	0	0	1.0	7.8	0.9	7.1	2.2	16.2	2.6	19.4
ME180 cells (0.3 cells)	162	6.5	0.2	3.2	0	0	0.6	8.6	0.4	5.5	2.4	36.2	2.5	37.7
HPV 18 IVT (30 copies)	162	9.0	0.7	7.3	0	0	0.7	7.2	0.8	8.3	2.3	25.3	2.6	28.5
HPV 16 IVT (30 copies)	162	10.8	0.1	0.8	0	0	0.1	1.3	0.4	3.8	0.9	8.4	1.0	9.3
HeLa cells (2.5 cells)	162	12.4	0	0	0.4	3.3	0.4	3.1	0	0	2.3	18.4	2.4	19.0
SiHa cells (1 cell)	162	7.5	0.3	3.7	1.0	13.0	0	0	0	0	4.8	63.6	4.9	65.0

SD = standard deviation; CV = coefficient of variation; IVT = in vitro transcript; S/CO = signal to cutoff ratio

^One sample had an invalid APTIMA HPV Assay result and was not included in the analyses.

Note: *Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.*

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Table 21: APTIMA HPV Assay Reproducibility Study 2: Signal Variability for Panel Members with Expected Positive Results

Panel Description (copies or cells/reaction)	n	Mean S/CO	Between Instruments		Between Operators		Between Lots		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
HPV 16 & HPV 18 IVT (100 copies)	162	23.2	0.4	1.5	0.6	2.3	0.8	3.4	0.8	3.4	1.5	6.3	2.0	8.4
SiHa cells (3 cells) & HeLa cells (7.5 cells)	162	18.6	0	0	1.7	9.3	0	0	3.5	18.6	3.7	20.0	5.4	28.9
HPV 18 IVT (100 copies)	160^	11.9	0.1	0.6	0.2	1.6	0.8	7.0	0.4	3.6	1.3	11.3	1.7	13.8
HPV 16 IVT (100 copies)	162	10.8	0	0	0.1	1.3	0	0	0.2	2.2	0.7	6.1	0.7	6.6
MS751 cells (1 cell)	162	13.6	0	0	0.6	4.3	0	0	2.5	18.4	2.1	15.2	3.3	24.2
ME180 cells (0.3 cells)	162	5.8	0	0	0.6	10.8	0.5	9.4	2.2	36.9	1.7	29.7	2.9	49.5
HPV 18 IVT (30 copies)	162	8.8	0.4	4.4	0.5	6.0	0.7	7.9	1.0	11.5	1.9	21.4	2.4	26.6
HPV 16 IVT (30 copies)	162	10.5	0	0	0.1	1.3	0.2	2.0	1.6	14.9	1.2	11.2	2.0	18.8
HeLa cells (2.5 cells)	159^	12.0	0.6	5.1	1.0	8.5	0	0	2.8	23.8	2.0	16.6	3.7	30.6
SiHa cells (1 cell)	162	7.4	0.9	12.5	0	0	0.7	9.3	1.8	24	4.2	56.8	4.7	63.8

SD = standard deviation; CV = coefficient of variation; IVT = in vitro transcript; S/CO = signal to cutoff ratio

^Five samples had invalid APTIMA HPV Assay results (2 for HPV 18 IVT (100 copies), 3 for HeLa cells (2.5 cells)) and were not included in the analyses.

Note: *Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.*

A third study was also conducted to determine assay reproducibility by testing a 6-member panel of pooled clinical PreservCyt specimens. Six unique pools of residual HPV-negative ThinPrep liquid cytology specimens were prepared as the matrix, two of which were tested as HPV-negative panel members. Four unique pools of HPV-positive ThinPrep liquid cytology specimens were used to prepare the low (n=2) and high (n=2) HPV-positive panel members. The low positive panel members had concentrations at the limit of detection of the assay (expected positivity: ≥ 95% determined for each individual HPV-positive pool from testing serial dilutions of the pools). The high positive panel members had concentrations at 1-2 logs above the estimated limit of detection for each individual HPV positive pool (expected positivity: 100% positivity). Each panel member was transferred (1 mL) into an APTIMA Specimen Transfer tube containing STM on the day of testing. Testing was conducted in-house by 2 operators using 1 reagent lot, 3 instruments, over 6 days (3 days for each operator), testing 2 runs per day in which the panel was tested in duplicate.

The panel members are described in Table 22, along with a summary of the agreement with expected results and analyte S/CO values at the 2.5th, 50th, and 97.5th percentiles of the signal distribution. The analyte S/CO variability for the panel members with expected positive results is shown in Table 23.

Agreement was 100% for the high HPV-positive panel members, ≥ 98.6% for the low HPV-positive panel members, and ≥ 94.4% were for the HPV-negative panel members.

Table 22: APTIMA HPV Assay Reproducibility Study 3: Panel Description, Percent Agreement, and Percentile Distribution of Analyte S/CO Values

Panel Description	% agreement (95% CI)	Analyte S/CO Percentile		
		2.5th	50th	97.5th
Low positive 1	98.6 (71/72) (92.5, 99.8)	1.5	10.1	19.3
Low positive 2	100 (72/72) (94.9, 100)	1.5	10.3	19.1
High positive 1	100 (72/72) (94.9, 100)	12.6	23.1	32.4
High positive 2	100 (72/72) (94.9, 100)	13.3	24.7	31.2
Negative 1	98.6 (71/72) (92.5, 99.8)	0	0	0.3
Negative 2	94.4 (68/72) (86.6, 97.8)	0	0	0.7

Table 23: APTIMA HPV Assay Reproducibility Study 3: Signal Analysis for Panel Members with Expected Positive Results

Panel Description	n	Mean S/CO	Between Instruments		Between Operators		Between Lots		Between Worklists		Within Worklists		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Low positive 1	72	9.8	0	0	0	0	0	0	2.2	22.8	3.0	30.4	3.7	38.0
Low positive 2	72	10.5	0	0	2.2	21.0	0.9	9.0	3.7	35.3	2.7	26.1	5.2	49.5
High positive 1	72	22.7	1.3	5.6	0	0	0.1	0.5	3.0	13.3	3.7	16.4	5.0	21.9
High positive 2	72	23.9	0	0	0	0	0	0	2.9	12.3	3.0	12.4	4.2	17.4

SD = standard deviation; CV = coefficient of variation; S/CO = signal to cutoff ratio

Note: *Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and CV are shown as 0.*

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Cross-Reactivity

The analytical specificity of the APTIMA HPV Assay was evaluated with PreservCyt solution media diluted 1:2.9 into STM and spiked with cultured bacteria, yeast, or fungi; cultured virus; or low-risk HPV in vitro transcripts. The organisms and test concentrations are identified in Table 24. The study criteria for assessing the effect of the presence of microorganism on the specificity of the assay were based on positivity. Cross-reactivity was observed with low-risk HPV genotypes 26, 67, 70, and 82, but not with any of the other organisms tested.

Table 24: Analytical Specificity Panel: Organisms and Concentration with No Cross-Reactivity

Organism	Test Concentration with No Cross-Reactivity	Organism	Test Concentration with No Cross-Reactivity
Bacteria			
Acinetobacter lwoffii	1×10^8 CFU/mL	*Listeria monocytogenes*	1×10^8 CFU/mL
Actinomyces israelii	1×10^8 CFU/mL	*Micrococcus luteus*	1×10^8 CFU/mL
Alcaligenes faecalis	1×10^8 CFU/mL	*Mobiluncus curtisii*	2×10^7 CFU/mL
Atopobium vaginae	5×10^7 CFU/mL	*Mycobacterium smegmatis*	1×10^8 CFU/mL
Bacillus cereus	1×10^8 CFU/mL	*Mycoplasma fermentans*	5×10^7 CFU/mL
Bacteroides fragilis	1×10^8 CFU/mL	*Mycoplasma genitalium*	1×10^8 CFU/mL
Bacteroides ureolyticus	1×10^8 CFU/mL	*Mycoplasma hominis*	5×10^7 CFU/mL
Bifidobacterium adolescentis	1×10^8 CFU/mL	*Neisseria gonorrhoeae*	1×10^8 CFU/mL
Bifidobacterium breve	1×10^8 CFU/mL	*Neisseria gonorrhoeae* and *Chlamydia trachomatis*	2.5×10^7 CFU/mL 2.3×10^5 $TCID_{50}$/mL
Campylobacter fetus-fetus	1×10^8 CFU/mL	*Neisseria meningitidis*	1×10^8 CFU/mL
Chlamydia trachomatis	3.2×10^5 $TCID_{50}$/mL	*Peptoniphilus lacrimalis*	1×10^8 CFU/mL
Clostridium difficile	6×10^7 CFU/mL	*Peptostreptococcus anaerobius*	1×10^8 CFU/mL
Clostridium perfringens	1×10^8 CFU/mL	*Propionibacterium acnes*	1×10^8 CFU/mL
Corynebacterium genitalium	1×10^8 CFU/mL	*Proteus mirabilis*	1×10^8 CFU/mL
Corynebacterium xerosis	1×10^8 CFU/mL	*Proteus vulgaris*	1×10^8 CFU/mL
Enterobacter cloacae	1×10^8 CFU/mL	*Providencia stuartii*	1×10^8 CFU/mL
Enterococcus faecalis	1×10^8 CFU/mL	*Pseudomonas aeruginosa*	1×10^8 CFU/mL
Escherichia coli	1×10^8 CFU/mL	*Ruminococcus productus*	1×10^8 CFU/mL
Finegoldia magna	1×10^8 CFU/mL	*Serratia marcescens*	1×10^8 CFU/mL
Fusobacterium nucleatum	1×10^8 CFU/mL	*Staphylococcus aureus*	1×10^8 CFU/mL
Gardnerella vaginalis	1×10^8 CFU/mL	*Staphylococcus epidermidis*	1×10^8 CFU/mL
Haemophilus ducreyi	1×10^8 CFU/mL	*Staphylococcus saprophyticus*	1×10^8 CFU/mL
Klebsiella pneumoniae	1×10^8 CFU/mL	*Streptococcus agalactiae*	1×10^8 CFU/mL

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Table 24: Analytical Specificity Panel: Organisms and Concentration with No Cross-Reactivity *(continued)*

Organism	Test Concentration with No Cross-Reactivity	Organism	Test Concentration with No Cross-Reactivity
Bacteria			
Lactobacillus acidophilus	$1x10^8$ CFU/mL	*Streptococcus pyogenes*	$1x10^8$ CFU/mL
Lactobacillus crispatus	$1x10^8$ CFU/mL	*Streptococcus sanguinis*	$1x10^8$ CFU/mL
Lactobacillus delbrueckii ssp. bulgaricus	$1x10^8$ CFU/mL	*Ureaplasma urealyticum*	$1x10^8$ CFU/mL
Lactobacillus jensenii	$1x10^8$ CFU/mL		
Yeast/protozoa			
Candida albicans	$1x10^8$ CFU/mL	*Trichomonas vaginalis*	$1x10^7$ cells/mL
Viruses			
Adenovirus 2	$1x10^7$ vp/mL	Herpes simplex virus 1	$2.5x10^5$ $TCID_{50}$/mL
Cytomegalovirus	$5.6x10^2$ $TCID_{50}$/mL	Herpes simplex virus 2	$5x10^4$ $TCID_{50}$/mL
Epstein-Barr virus	$4.3x10^6$ vp/mL	SV40	$1.2 x10^4$ $TCID_{50}$/mL
HIV-1	$1.0x10^6$ copies/mL		
Non-targeted HPV genotypes			
HPV 6	$2.5x10^6$ copies/mL	HPV 61	$2.5x10^6$ copies/mL
HPV 11	$2.5x10^6$ copies/mL	**HPV 67**	1 copy/mL
HPV 26	2.5 copies/mL	HPV 69	$2.5x10^6$ copies/mL
HPV 30	$2.5x10^6$ copies/mL	**HPV 70**	1 copy/mL
HPV 34	$2.5x10^6$ copies/mL	HPV 71	$2.5x10^6$ copies/mL
HPV 42	$2.5x10^6$ copies/mL	HPV 73	$2.5x10^6$ copies/mL
HPV 43	$2.5x10^6$ copies/mL	HPV 81	$2.5x10^6$ copies/mL
HPV 44	$2.5x10^6$ copies/mL	**HPV 82**	2.5 copies/mL
HPV 53	$2.5x10^6$ copies/mL	HPV 85	$2.5x10^6$ copies/mL
HPV 54	$2.5x10^6$ copies/mL		

vp = viral particles CFU = colony forming units $TCID_{50}$ = tissue culture infective dose 50

Note: *Bold indicates types where cross-reactivity (> 5% positivity) was observed when tested at concentrations greater than that noted in the table.*

The analytical sensitivity of the APTIMA HPV Assay in the presence of microorganisms was evaluated with the same panel described in Table 24, which was also spiked with a low concentration of HPV infected SiHa cells (1 cell per reaction). The study criteria for assessing the effect of the presence of microorganism on the sensitivity of the assay were based on positivity. The sensitivity of the APTIMA HPV Assay was not affected by any of the organisms tested.

Interference

The substances described in Table 25 were individually spiked into PreservCyt solution at 1% and 10% v/v or w/v, diluted with STM and then tested in the APTIMA HPV Assay. All substances were tested in the presence and absence of HPV infected cultured cells (SiHa, 3 cells/reaction). Interference was observed with two of the seven lubricants that contained Polyquaternium 15, and one of the five anti-fungal medications that contained tioconazole. Interference was not observed with any of other substances tested.

Table 25: Substances Tested for Possible Interference with the APTIMA HPV Assay

Product Category	Product Brand or Type	Highest Concentration* Tested that Did Not Interfere with Assay Performance
Lubricant	KY Sensual Mist	10% v/v
	KY Warming Jelly	10% w/v
	KY Warming Liquid	10% v/v
	CVS Brand Personal Lubricant	10% w/v
	Target Brand Warming Massage Lotion and Personal Lubricant	10% v/v
	Astroglide Personal Lubricant	0.3% w/v (0.075% w/v test sample)
	Target Brand Lubricating Liquid	0.1% v/v (0.025% v/v test sample)
Spermicide	Gynol II Vaginal Contraceptive Original Formula	10% w/v
	Gynol II Vaginal Contraceptive Extra Strength	10% w/v
	Delfen Vaginal Contraceptive Foam	10% w/v
	Encare Vaginal Contraceptive	10% w/v
	Conceptrol Vaginal Contraceptive	10% w/v
Anti-fungal/ Anti-Itch Medication	Vagisil Maximum Strength	10% w/v
	Monistat Soothing Care	10% w/v
	Monistat 3 Combination Pack	10% w/v
	Target Brand Tioconazole 1	0.3% w/v (0.075% w/v test sample)
	Target Brand Miconazole 3	10% w/v
Glacial Acetic Acid	EMD M/N AX0073-11	10% v/v
Whole Blood	whole blood	10% v/v

*Concentration in the PreservCyt Solution specimen; test sample concentration is 1/4 the concentration due to transfer of PreservCyt solution specimen into transfer tube containing STM.

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